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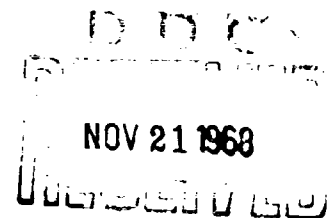
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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

QUANTITATIVE STUDY OF THE GRANULOPECTIC ACTIVITY OF
THE RETICULOENDOTHELIAL SYSTEM BY INTRAVENOUS INJECTION
OF INDIA INK INTO VARIOUS ANIMAL SPECIES

1. Method of Quantitative Study of the Granulopectic Activity
of the Reticuloendothelial System by Intravenous Injection
of Carbon Particles of Known Dimensions

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B. N. Halpern, G. Biozzi,
G. Mene and B. Benacerraf

After the reticuloendothelial system was defined and characterized by the studies of Aschoff and Kyono [1], numerous investigations were carried out in order to study the activity and function of this system. To demonstrate the bacteriophagic activity of the reticuloendothelial system, the various authors used either solutions of vital stains [2] or suspensions of particles, such as colloidal silver, manganese, India ink, etc [2, 3, 4, 5, 6]. The methods involving the intravenous injection of India ink are used for studying, more particularly, the granulopectic activity of the cells of the reticuloendothelial system of the vascular bed in direct contact with the blood, since the carbon particles which are generally employed are incapable of crossing the walls of the vessels, and are not eliminated by the excretory organs. However, the authors who used India ink have injected suspensions of carbon particles of which they knew neither the size nor the degree of dispersion. The fate of the India ink as a function of the quantity of ink injected has never been subjected to a special study; the mode of distribution of the ink in the various organs according to the animal species has never been investigated from the quantitative point of view. The various authors were content with studying the distribution of the carbon in the organs by histological methods, without quantitatively determining either the rate of

disappearance of the carbon particles from the blood or their accumulation in the organs. It is precisely because of these large number of variables that it is impossible to draw from these experiments any valid conclusions regarding the real activity of the reticuloendothelial system.

The object of the present work is to study the effects of the intravenous injection of an ink suspension which is well defined both as to weight and the degree of dispersion, and to establish a quantitative method which permits the functional exploration of the reticuloendothelial system as well as the definition of the limits within which this method is applicable. This technique is based on the quantitative study of the kinetics of disappearance of the carbon particles from circulation, and of their distribution in the organs after an intravenous injection of India ink.

In this paper we shall report the results obtained with only two animal species: the rat and the mouse.

EXPERIMENTAL STUDY

A. Kinetics of Disappearance of the Carbon Particles from Circulation. -- a) In the rat. -- Technique: We used the India ink commercially sold under the name "Pelikan." Examination of this ink under the electron microscope showed that it is perfectly dispersed and that it contains particles of the same size, about 200 Å (Fig. 1)¹.

This ink contains 80 mg of carbon per milliliter. Since Wright's studies [7] have shown the importance of a protective colloid in the maintenance of the perfect dispersion of the ink in vivo, the latter was diluted in physiological saline containing 1% of gelatine for ink dilutions of up to 30%, and 2% gelatine for more concentrated solutions. Variable carbon doses, expressed in milligrams of carbon per 100 g of animal weight were injected in the caudal vein of the rat. For the doses of 8, 16 and 32 mg of carbon per 100 g we used a 20% dilution of our ink, and for the doses of 48 and 64 mg of carbon per 100 mg a dilution of 30% and 40%, respectively.

To check the stability and dispersion of the ink particles in the presence of blood, we carried out control tests in vitro; these tests showed that within the concentration limits employed the particles remained in stable suspension both in the serum and in the citrated blood.

¹We are greatly indebted to Dr. Pierre Lepine, Head of the Virology Service of the Pasteur Institute, for having prepared this photograph in his Service.

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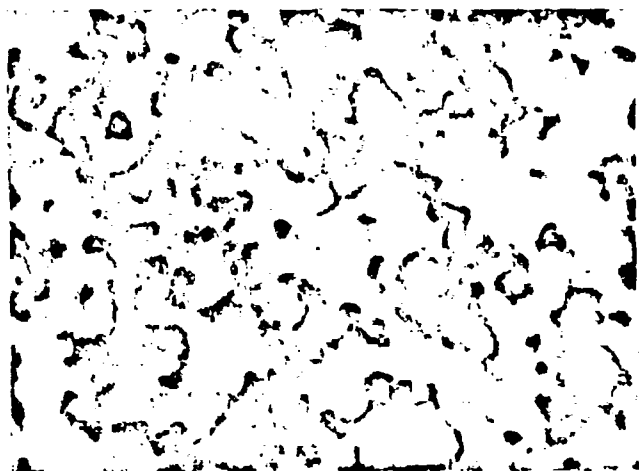


Fig. 1. Microphotograph, Obtained by Electron Microscopy, of the India Ink Employed. Magnification: x 55,000. (Virology Service of the Pasteur Institute).

To check the stability and dispersion of the ink particles in the presence of blood, we carried out control tests in vitro; these tests showed that within the concentration limits employed the particles remained in stable suspension both in the serum and in the citrated blood.

To study the kinetics of the disappearance of the ink from the blood, samples were taken by puncturing the retroorbital venous plexus, which communicates with the cavernous sinus of the rat, by means of a glass micropipette calibrated for 0.025 ml, previously washed with an anticoagulant solution (Liquoid or heparin). The plexus can be readily reached in this animal species by introduction of a fine glass pipette into the internal angle of the ocular orbit (Figs. 2 and 3). After perforation of the palpebral sac one reaches the maxilla which covers the ophthalmic venous plexus, and then the blood rises in the pipette by capillary action². By this method numerous samples may be taken at precise times; the method is perfectly well tolerated by the animal.

The blood sample is laked in 2 ml of 0.1% sodium carbonate solution, and the carbon is determined by means of Meunier's electrophotometer in red light. The carbon content of the blood

²The details of this technique will be published at a later date.

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is determined by comparison with a calibration curve. The following amounts of carbon were injected: 8, 16, 32, 48 and 64 mg per 100 g of body weight of the animal. The blood samples were collected 2, 4, 6, 10, 20, 35 and 50 minutes after the injection of the ink. Four hundred and seventy-four white rats whose weight ranged from 110 to 140 g, were used in these tests.



Fig. 2. Blood-Sampling Technique by Puncturing the Sinus by Means of a Calibrated Pipette, in the Rat.

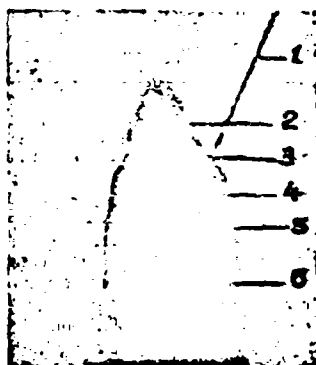


Fig. 3. X-ray Picture of the Cranium of the Rat, Showing the Position of the Pipette at the Moment of the Venous Puncture. 1 -- Pipette; 2 -- Incisors; 3 -- Malar process of the maxilla; 4 -- End of pipette; 5 -- Zygomatic arch; 6 -- Tympanic bulla.

Results. -- The animals have tolerated the injections of India ink perfectly well, and displayed no immediate

disturbance¹, with the exception of the dose of 64 mg per 100 g, which caused a state of temporary shock, with generalized tremors in the animal.

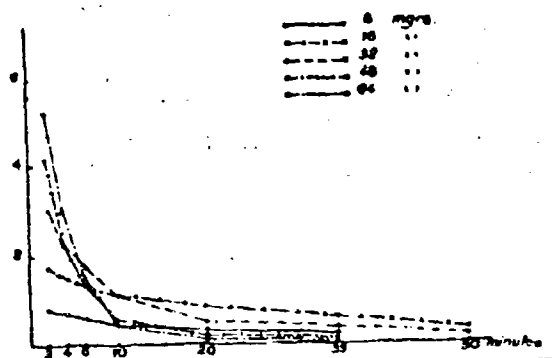


Fig. 4. Kinetics of the Disappearance of Carbon Particles of 200 Å from the Blood, Injected Intravenously in the Rat, as a Function of the Injected Dose.

The ordinates represent the concentration of carbon particles in the blood in mg/ml; the abscissas the time in minutes.

The carbon concentrations in the blood, as a function of the time and the dose of ink injected, are plotted in Fig. 4 as the arithmetic averages of the results obtained for each dose. For the doses of 8 and 16 mg of carbon per 100 g only a small number of animals exhibit values that are too far removed from the average; in these animals it was always found, on autopsy, that the weight of their organs (liver and spleen) did not correspond to their size.

It can be seen from Fig. 4 that with the dose of 8 mg per 100 g the disappearance of the carbon from circulation takes place in a slow and gradual manner. The curve assumes a regular parabolic shape. With the dose of 16 mg per 100 g the disappearance of the carbon particles from the blood is essentially parallel to the preceding curve, but displaced in height, and the ink remains in circulation for a longer period of time. However the shape of the curve changes completely when we inject a dose that is twice the previous one, i.e., 32 mg per

¹While it is true that the animals do not display any immediate disturbances, it should be stressed that a high tardive mortality rate is observed in the case of the dose of 32 mg per 100 g, and that the mortality attains 100% with the dose of 48 mg per 100 g and above. Death occurs between the 14th and 24th hour after the injection, and we are not able, as yet, to determine the cause of this death.

100 g. The rate of disappearance of the India ink accelerates considerably, and the cleansing of the blood occurs at a much faster rate than with the lower dose. This acceleration of the disappearance of the India ink from circulation increases even further with the larger doses, viz., 48 and 64 mg per 100 g. With the dose of 64 mg per 100 g, for example, only 0.45 mg of carbon is found per milliliter of blood ten minutes after the injection, while in the case of the injection of 16 mg of carbon per 100 g (i.e., a dose one-fourth as great) the carbon concentration ten minutes after the injection is still 1.12 mg per milliliter of blood.

Thus it can be seen that in the rat the rate of disappearance of carbon from circulation exhibits a completely individual and apparently paradoxical behavior: the larger the amount of carbon injected the faster it disappears from the blood.

Later on we shall give an explanation for this unexpected behavior.

b) In the mouse. -- Technique: In this animal species we used the same methods as in the rat. The amounts of carbon injected were 8, 16, 24, 32, 48 and 64 mg per 100 g of animal body weight. These doses correspond to those used in the rat. The ink was diluted in physiological saline containing 1% gelatine; the dilution was 1:10 for the 8 mg dose and 1:5 for the higher doses. The study was carried out on 150 mice, whose weight varied between 15 and 25 g.

Results. -- The animals tolerated the injections of India ink perfectly well, and exhibited no immediate disturbances, except for the 48 and 64 mg doses which caused the same troubles as in the rat. In this animal species, too, ink doses greater than 24 mg per 100 g -- though tolerated without immediate trouble -- caused a mortality rate of close to 100% within 24 hours following the injection.

The rate of disappearance of carbon from circulation in the mouse after the intravenous injection of increasing doses of India ink are schematically shown by the curves of Fig. 5.

Thus by injecting increasing doses of carbon not only do we increase the carbon concentrations in the blood but we also considerably increase the duration of the presence of the ink in circulation. In this respect we note that in the mouse the mode of circulatory cleansing at the doses ranging from 8 to 32 mg per 100 g is considerably different from that at doses greater than 32 mg per 100 g. Thus in the case of the 8 mg

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dose the carbon concentration in the blood attains 0.54 mg per ml 2 minutes after the injection. At this dose the ink almost completely disappears from circulation 20 minutes after the injection. In the case of the 16 mg dose the concentration of carbon in the blood 2 minutes after the injection is 1.50 mg per ml, and the ink disappears from circulation only after approx. 50 minutes. In the case of the 24 and 32 mg doses the carbonemia curves are essentially parallel to the previous curves, though displaced in height.

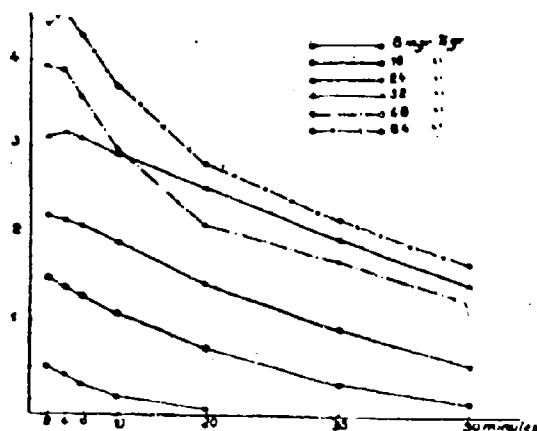


Fig. 5. Rate of Disappearance, from the Blood, of Carbon Particles of 100 Å [sic], Injected into the Mouse Intravenously, as a Function of the Injected Dose.
Ordinate: Carbon concentration in blood in mg/ml; Abscissa: Time in minutes.

However the shape of the curve of circulatory cleansing changes when we inject a carbon dose of 48 mg per 100 g. At this dose we note an acceleration of the cleansing, whose result is a faster drop of carbonemia than in the case of the lower dose. The same phenomenon is observed in the case of the 64 mg dose. Thus the phenomenon of an accelerated disappearance of carbon from the blood when a high dose of carbon is injected -- a phenomenon which we have described in the case of the rat -- can also be observed in the mouse. However in the mouse this phenomenon is provoked only with doses that are more than twice as large as those which are required to obtain this phenomenon in the rat. Furthermore, the behavior of the mouse in this respect is characterized by a great irregularity from one animal to the other. Not only is the phenomenon of accelerated disappearances from the blood more attenuated in this animal species than in the rat, but it is also much more irregular; even at the doses which call forth this phenomenon, certain animals

display it and some do not. This irregularity in the response of the mouse contrasts with the highly uniform behavior of the rat.

B. Distribution of Carbon in the Organs. -- We considered the study of the distribution of India ink in the organs to be an indispensable corollary for completing our knowledge of the fate of the carbon particles upon their disappearance from circulation.

Technique: The distribution of the injected India ink in the animal's organism was studied quantitatively through the recovery and measurement of the weight of the carbon in the following organs: liver, spleen, lungs and kidneys. This determination was made at the same ink doses as were used for the study of the rate of disappearance of the carbon particles from the blood. In these studies the animals were sacrificed only after the ink had completely disappeared from circulation. The animal was bled to death by sectioning of the carotids; the organ in question was removed and carefully weighed; then it was introduced into an NaOH solution (10 ml solution per gram of fresh organ). Next, 15 ml of 95% alcohol were added per gram of organ. The mixture was brought to boil over a 15 minute period. At that time the hydrolysis of the organ should be complete.

In this way two superimposed layers are obtained: a lower layer, which contains the India ink, and an upper alcoholic layer stained by the pigments, which does not contain any trace of the ink. After cooling, the upper layer is drawn off. Then 5 ml of 95% alcohol are added per gram of organ. The mixture is again boiled for 10 minutes and the alcohol is eliminated as above. This last operation is repeated four times. Then the solution containing the India ink is treated with 15 ml of distilled water per gram of organ and with concentrated HCl until a pH of approximately 1 is obtained. The ink agglutinates and settles to the bottom. After centrifuging, the supernatant liquid is rejected and the precipitate is washed with acidified water. The supernatant is again rejected and the residue is taken up in the same volume of alcohol, then twice taken up in the same volume of acetone. The residue is dried in the oven at 100° until a constant weight is obtained, and after prior weighing of the tube, the quantity of carbon in the organ is determined.

To check the accuracy of our technique, we carried out about 20 tests in vitro, adding known quantities of India ink to the organs, and subjecting them to the same operations. In all cases we recovered 98-100% of the carbon added.

In the rat the carbon was determined individually in each animal. Altogether 30 rats were investigated, or 6 rats per dose.

In the mouse, since the amounts of carbon found in the organs were too small to permit a gravimetric determination with the organs of a single animal, we carried out these determinations on the organs of three animals, mixed together.

Simultaneously we investigated, in these animals, the histological aspect of the organs, in order to establish the cellular topography of the distribution of the carbon. Thus the liver, spleen, lungs and kidneys were examined histologically.

Results: The results obtained in the rat are shown in Figs. 6 and 7. With an ink dose of 8 and 16 mg of carbon/100 g only the spleen and the liver (and the bone marrow, whose carbon content could not be determined due to practical difficulties) accumulate carbon granules. The liver and the spleen share, between them, about 90% of the ink injected. At the 32 mg dose the spleen can no longer increase its granuloplectic ability under these experimental conditions; it attains its maximum concentration which is about 2 to 3 mg per gram.

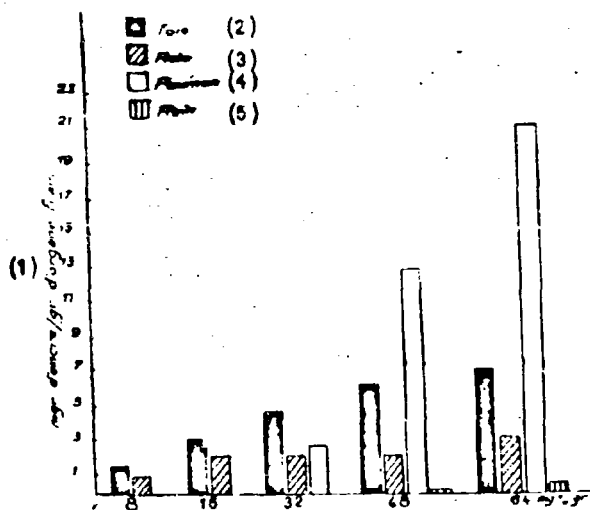


Fig. 6. Distribution of Carbon in Mg per Gram of Fresh Organ, in the Various Organs of the Rat, as a Function of the Injected Dose.

1 -- Mg of ink per g of fresh organ; 2 -- Liver; 3 -- Spleen; 4 -- Lung; 5 -- Kidney.

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The liver increases its absorption capacity but not in proportion to the amount of carbon injected. The lung, which did not contain measurable quantities of carbon for the preceding doses, retains at the dose of 32 mg per 100 g an amount of carbon which is more or less equal to that of the spleen.

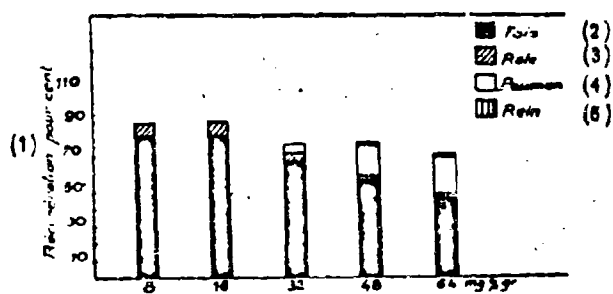


Fig. 7. Total Percentage and Percentage of the Carbon Recovered in the Various Organs of the Rat as a Function of the Injected Dose.

1 -- Recovery in %; 2 -- Liver; 3 -- Spleen; 4 -- Lung; 5 -- Kidney.

For the dose of 43 mg per 100 g the splenic concentration remains stationary, while the liver tends to increase its coefficient of granulopexis. This time it is the lung that appears to have the highest carbon concentration, if the latter is expressed in terms of weight per gram of fresh organ.

In the last stage, which corresponds to the dose of 64 mg/100 g, the spleen and the liver seem to have attained their maximum granulopexis power, while the participation of the lung increases considerably until it reaches the enormous concentration of 21 mg per gram of fresh organ. At this very high dose one also finds a small quantity of carbon (less than 1 mg per gram) in the kidney.

The results obtained in the mice are different from those of the rat, as can be seen from Figs. 8 and 9. If we consider the distribution of the India ink in the organs of this animal, we note that at the 16 mg dose the liver and the spleen retain 2.4 mg of carbon per gram, which represents 92.1% of the ink injected, while the lungs do not contain any. At twice the former dose of carbon the lungs accumulate only 4% of the carbon injected (1.15 mg per gram), while the hepatosplenic area of the reticuloendothelial system fixes 60.3% of the injected ink, which corresponds to a concentration of 3.85 mg of carbon per gram of fresh organ.

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Fig. 8. Distribution of Carbon in Mg per G of Fresh Organ, in the Various Organs of the Mouse, as a Function of the Injected Dose.

1 -- Carbon, mg/g of fresh organ; 2 -- Liver and spleen; 3 -- Lung; 4 -- Kidney.

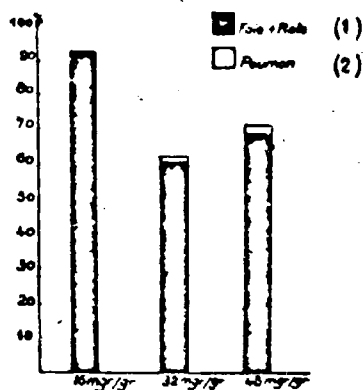


Fig. 9. Total Percentage and Percentage of Carbon Recovered in the Various Organs of the Mouse, as a Function of the Injected Dose.

1 -- Liver and spleen; 2 -- Lung.

At the 48 mg dose we recover more or less the same total percentage in the examined organs. The lungs do not seem to be able to fix more carbon, while the carbon content of the liver and the spleen increases considerably. We have found only traces of carbon in the kidney, even in the case of high doses.

The histological study carried out in the rat shows that for doses of 8 and 16 mg per 100 g the carbon particles are fixed by the macrophages of the liver, i.e., the Kupffer cells, and those of the spleen, and notably the reticular cells. In the reticular cells of the spleen, the India ink is accumulated in the form of fine granulations, an appearance which is in no

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way comparable to that of the coarse grains encountered on the level of the Kupffer cells. Sections of the lung and kidney do not exhibit detectable amounts of carbon at these doses (Figs. 10, 11 and 12).

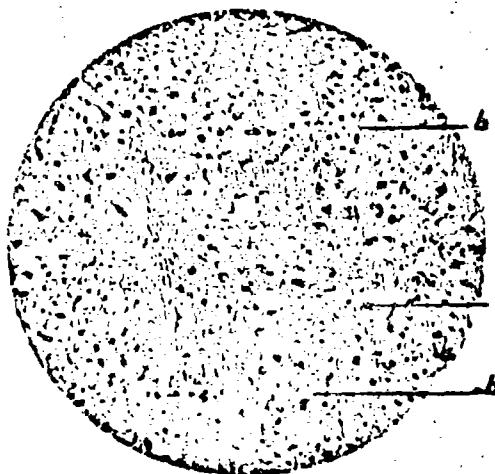


Fig. 10. Liver of a Rat Treated with an Intravenous Injection of 16 mg of Carbon per 100 g of Body Weight. Animal sacrificed 1 hour 30 minutes after injection. Presence of carbon in the intertrabecular sinus (a). Photograph shows the macrophagia of the carbon granules by Kupffer's cells.

In the case of higher doses, the macrophagia of the liver and the spleen is more pronounced and in the liver it tends to assume, starting with the 32 mg dose, an essentially periportal topography (Fig. 13). In the spleen the macrophages which have subjected the carbon to phagocytosis form a crown around the red pulp, while the cells of the white pulp contain practically no such crown (Fig. 14). The behavior of the lung in this respect is quite peculiar: with carbon doses of up to 16 mg we note only traces of carbon on the histological sections of this organ. However, the carbon concentration increases as the ink dose injected is increased. The carbon content of the lung, which is very notable already at the 32 mg dose (Fig. 15), is considerable at the dose of 48 and 64 mg of carbon per 100 g.

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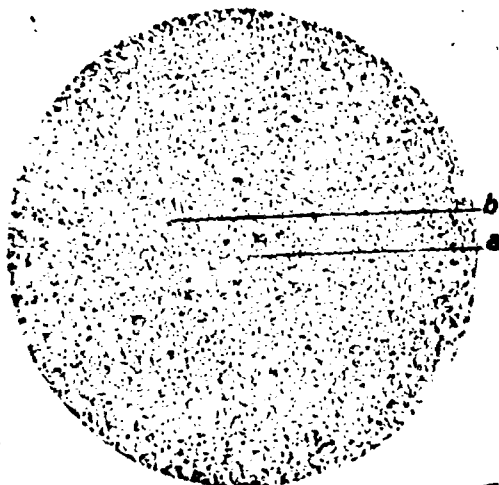


Fig. 11. Spleen of a Rat Treated with an Intravenous Injection of 16 mg of Carbon per 100 g. Animal sacrificed 1 hour 30 minutes after injection. Presence of a relatively small amount of carbon in the red pulp (a). The grains are situated in the interior of the reticular cells. A few carbon particles are fixed on the red blood cells.



Fig. 12. Lung of a Rat Treated with 16 mg of Carbon per 100 g of Body Weight, Administered by Intravenous Injection. Animal

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sacrificed 1 hour 30 minutes after injection. Absence of ink in lungs.

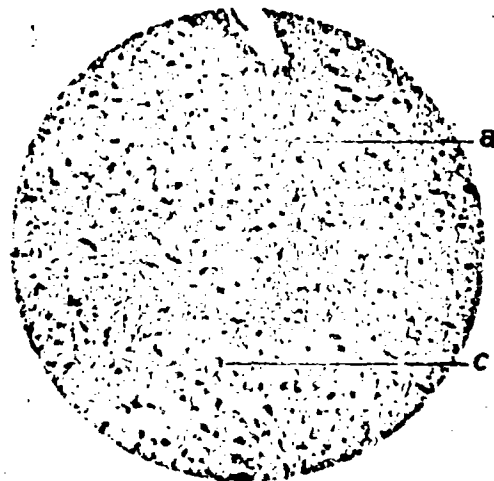


Fig. 13. Liver of a Rat Treated with 32 mg of Carbon per 100 g of Body Weight, Administered by Intravenous Injection. Animal sacrificed 1 hour 30 minutes after injection. Presence of considerable amount of carbon in the intertrabecular sinuses (a).

Macrophagia of the granules by the Kupffer cells.

The histological analysis of these lung sections⁶ reveals that the greater part of the carbon fixed in the lung is to be found in the capillaries; the conglomerated carbon particles seem to adhere to the capillary wall without causing either embolism of a complete obliteration of the lumen. A small portion of the carbon was localized in the interalveolar cells, and even in the cells of the alveolar lining.

Although it is impossible to say, with certainty, how the carbon particles adhere to the walls of the pulmonary capillaries, it can nevertheless be stated that they are certainly not free in the circulatory stream.

DISCUSSION

The investigations carried out until now on the fate of India ink in the organism had always been based on histological

⁶At this point we would like to express our thanks to Prof. Jean Verne of the Faculty of Medicine of Paris, for his courtesy in interpreting these sections for us in the light of his great competence in this field.

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methods which do not permit any quantitative evaluation -- even an approximate one -- of the phenomenon. So far all that has been determined was the distribution of the carbon granules in the different cells of the organism, without taking into consideration the doses of ink injected and the size or homogeneity of the carbon particles which, however, seem to represent essential factors. This explains the lack of numerical data on the granuloplectic power of the organs themselves as a function of the carbon doses injected or of the dispersion or stability of the suspension.

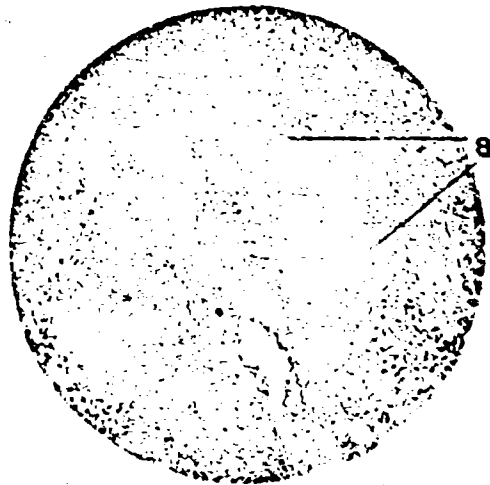


Fig. 14. Spleen of a Rat Treated with 32 mg of Carbon per 100 g of Body Weight, Administered by Intravenous Injection. Animal sacrificed 1 hour 30 minutes after injection. Presence of carbon granules clearly visible in the reticular cells of the red pulp whose boundaries are outlined by these granules. Note the absence of carbon in the cells of the white pulp.

Dispite this fact, however, numerous authors have restore^d to injecting India ink with a view to blocking the reticuloendothelial system, in order to determine the latter's role in the various physiopathological states such as occur in anaphylaxis, during the production of antibodies or during experimental infections, without standardizing either the doses employed, or the experimental conditions, and without even verifying whether the reticuloendothelial system could really be blocked.

The quantitative methods employed in this work make it possible to determine, with certainty, how the reticuloendothelial system behaves under the experimental conditions defined

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in this study, and for particles of a given size. These methods make it possible to obtain consistent and reproducible results.

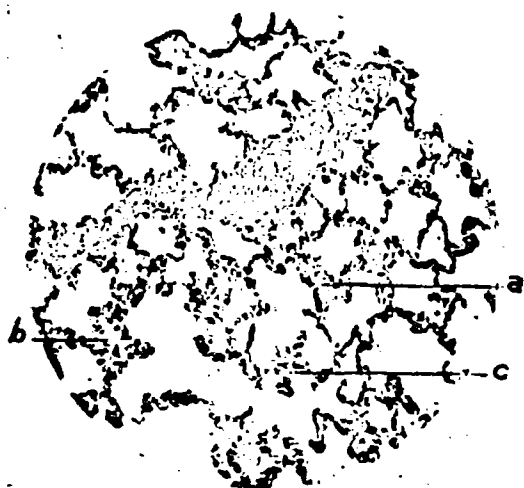


Fig. 15. Lung of a Rat which Had Been Intravenously Injected with 32 mg of Carbon per 100 g of Body Weight. Animal sacrificed 1 hour 30 minutes after injection. Presence of relatively large amount of carbon in the capillaries where the granules seem to be conglomerated (a). We also note small quantities of carbon in the pulmonary stroma (b) and in the cells of the alveolar lining (c).

The study carried out, in the rat, on the disappearance of the India ink from the blood stream and its distribution in the organs shows that the fate of the carbon varies according to the dose employed. Thus in the case of doses of 8 and 16 mg of carbon per 100 g of body weight we note a regularly gradual disappearance of the ink from the blood and its corresponding accumulation in the macrophages of the liver and the spleen, with the ink disappearing more rapidly from the blood in animals which had received 8 mg of carbon per 100 g than in animals which had received 16 mg of carbon per 100 g. At these doses the lung and the kidney contain practically no carbon granules.

For doses greater than 16 mg of carbon per 100 g we note that the rate of disappearance of the carbon particles from the blood is considerably accelerated and that the circulatory cleansing takes place at a faster rate than at the lower dose. Simultaneously we note that large amounts of carbon deposit in

the lung. At these doses the kidney also contains only small amounts of carbon.

The histological sections prepared from the organs of animals sacrificed after the complete disappearance of the ink from circulation show that in the liver and the spleen the carbon granules are stored in the reticuloendothelial cells by a veritable act of macrophagia. As far as the lung is concerned, its behavior varies according to the dose of carbon injected: the lung does not retain any carbon up to the dose of 16 mg. In this respect its activity is considerably different from that of the hepatosplenic and hemopoietic areas. Above the dose of 16 mg per 100 g we note that the lung fixes some carbon, but it seems that the greater part of the carbon is retained in the capillaries by a very special mechanism, and that only a very small part undergoes phagocytosis by the histiocytes and the cells of the alveolar lining. Our observations relating to the behavior of the lung toward the carbon cells may be compared with the facts reported by Roger, Binet and Verne regarding the fate of fat droplets retained in the pulmonary capillaries after intravenous injections of oil. These authors maintain that the fat droplets are first fragmented by the hypertrophied endothelial cells and then digested by a special mechanism to which they gave the name "pulmonary lipodieresis."

How are we to explain this phenomenon which appears in the mouse only at much higher doses and in a much more attenuated form? The possibility of the intervention of a serial factor causing the flocculation of the ink when the latter is injected at high doses has been ruled out by the negative outcome of experiments carried out in vitro with serum or with citrated plasma. Other hypotheses, such as the existence of a hormonal control of the granulopexis which establishes a hierarchy of the activity of the organs according to the carbon concentration of the blood have not been borne out by experiments.

The fact remains that in the case of the rat, for doses greater than 16 mg of carbon per 100 g, the disappearance of the carbon from the blood is accelerated with increasing doses and at the same time we note a concomitant fixation of the carbon particles in the lung and the kidney. Do these doses provoke a sudden peptic activity of the reticuloendothelial cells of the lung in the case of carbon particles of approximately 200 Å, an activity similar to that possessed by the Kupffer cells of the liver and the reticular cells of the spleen? Or do we have to do in the case of these ink doses with a new factor which determines the uptake of carbon by the lung? This problem has been made the object of a study which will be reported in a subsequent publication and which sheds some light on these questions.

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The presence of a new factor intervening in the disappearance of the carbon particles from the blood for doses greater than 16 mg per 100 g is confirmed by the examination of the results shown in Fig. 16, where the carbon concentrations of the blood are expressed in logarithmic values. We note that for the doses of 8 and 16 mg of carbon per 100 g, the rate of disappearance of the carbon particles from the blood is a linear function, while at higher doses the shape of the "disappearance" curve is irregularly parabolic.

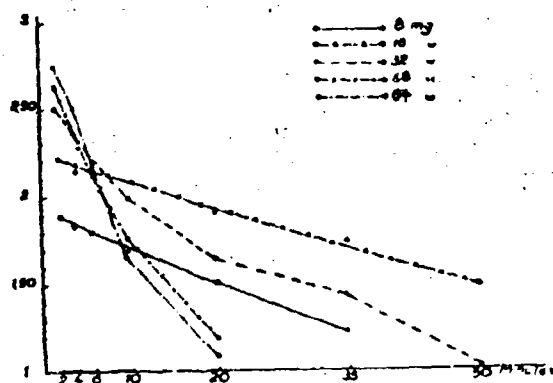


Fig. 16. Rate of Disappearance from the Blood of Carbon Particles of 100 Å [sic] Injected Intravenously to the Rat in Doses of 8, 16, 32, 48 and 64 mg/100 g, Expressed in Logarithm of the Concentration in the Blood.

Thus the injection, to the rat, of carbon doses greater than 16 mg per 100 g introduces a new factor into the mechanism of circulatory cleansing. These doses cannot be used for the quantitative exploration of the granuloplectic function of the liver and the spleen.

The quantitative exploration of the granuloplectic function of these organs may be carried out without committing an error by using doses of less than 16 mg per 100 g. Under these conditions only, the curves showing the disappearance of the carbon particles from the blood, their gravimetric distribution in the organs, give a good reflection of the granuloplectic activity of the liver and spleen for particles of 200 Å. The described technique may be used for an exact study of the influence of the different factors on the granuloplectic function of the liver and the spleen. To demonstrate the influence of these factors and to assign them a meaningful value, it is necessary to establish first the curve of the dispersion of the points and to calculate the standard deviation statistically. By using

the classical formula

$$--\sqrt{\frac{10}{n}}$$

we have calculated the standard deviation for the 8 and 16 mg dose of carbon in the rat (Fig. 17) and for the 16 mg dose in the mouse (Fig. 18). Only those modifications will have a meaningful value which are situated outside the limits of spontaneous variations.

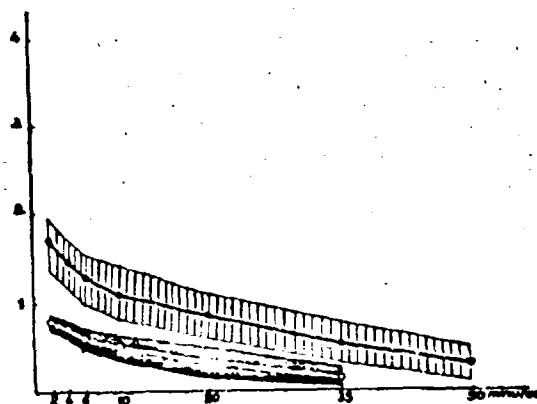


Fig. 17. Standard Deviation and Mean Values of the Carbon Concentrations in the Blood after Intravenous Injection of 8 and 16 mg of Carbon per 100 g in the Rat. The standard deviation was calculated by the formula:

$$--\sqrt{\frac{10}{n}}$$

A supplementary verification of the method was carried out for the dose of 16 mg of carbon per 100 g. After injecting this dose of ink in rats, these animals were sacrificed after varying periods of time and the carbon was determined in the blood, the liver and the spleen. The results are shown in Table I. They show that, for this dose, at least 90% of the injected carbon is recovered in a state distributed between the blood and the organs examined, while the remaining 10% of carbon would be found in the bone marrow and the other areas not included in these experiments.

The same results were observed in the mouse.

NOT REPRODUCIBLE

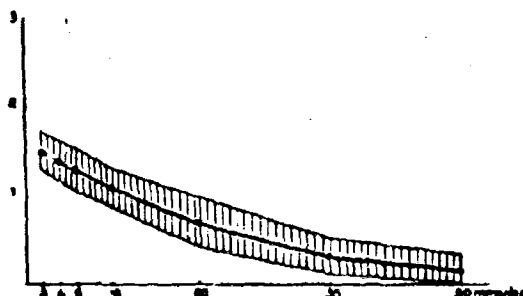


Fig. 18. Standard Deviation and Mean Values of the Carbon Concentrations in the Blood After Intravenous Injection of 16 mg of Carbon per 100 g in the Mouse.

Table I.

Table Showing the Mean Distribution of India Ink in the Blood and the Various Territories of the Reticuloendothelial System in the Rat

(1) Temps après l'injection en minutes	(2) Sang (mg)	(3) Foie (mg)	(4) Rate (mg)	(5) Ink injectée (mg)	(6) Total l'encrasse- ment (mg)	(7) Perte (mg)	(8) Pourcentage de l'encrasse- ment
0	11,2	3,8	0,4	17,1	15,3	1,8	89,6
10	8,3	2,9	0,6	19,8	18,7	1,1	94,4
20	4,3	13,8	1,3	20	19,4	0,6	97
60	0	15,6	1,7	19,8	17,3	2,5	87,4

(9)
(10)

(9) La masse sanguine chez le rat est évaluée à 6,3 cm³ p. 100 g.
(10) Il s'agit de l'organe entier.

1 -- Time after injection, minutes; 2 -- Blood; 3 -- Liver; 4 -- Spleen; 5 -- Ink injected; 6 -- Total ink recovered; 7 -- Loss; 8 -- Percentage of ink recovered; 9 -- The amount of blood in the rat is estimated at 6.3 cm³ per 100 g; 10 -- Refer to whole organ.

However, when the carbon doses are increased, we note that the recovery shows a discrepancy which increases with increasing carbon doses injected (Figs. 7 and 9). This shortage attains as much as 30% in the extreme dose of 64 mg of carbon per 100 g in the rat and 40% in the mouse. Where is the absent carbon fixed? It is probable that the hemopoietic and lymphatic organs increase their storage coefficient, but it is little likely that these relatively small organs can accumulate such large amounts of carbon. Hence it must be assumed that certain vascular areas of other tissues -- skin, muscle, digestive tract, etc. -- retain a certain amount of ink by the same mechanism as the lung.

In the mouse -- by contrast to the rat -- the disappearance of the carbon from the blood stream takes place in a gradual and regular manner until the dose of 32 mg of carbon per 100 g of body weight, as may be seen from the analysis of Fig. 5. It is only with the doses of 48 and 64 mg per 100 g that we note an acceleration of the circulatory cleansing which is reflected by a more inclined slope of the carbonemia curves. Nevertheless, the factor of pulmonary fixation, which is a determining factor in this respect in the rat, plays only a minor role in the mouse, as is shown by the slight difference between the carbon contents of the lung at 32 and 48 mg per 100 mg, and at the same time by the slight inclination of the slopes of the "disappearance" curves.

It is possible, in the study of the granuloplectic function of the hepatic and splenic reticuloendothelial system, to use relatively higher doses of India ink in the rat.

CONCLUSIONS

1. We have described a method permitting the quantitative evaluation of the granuloplectic activity of the reticuloendothelial system in contact with the blood in various animal species. This method consists in introducing, intravenously, a homogeneous and standardized suspension of carbon, and to simultaneously study the kinetics of its disappearance from the blood and its accumulation in the various organs by means of quantitative measurements.

2. In the rat, during the injection of carbon doses of up to 16 mg per 100 g, the disappearance of the carbon from the blood takes place as a direct function of the injected dose. For these doses only the hepatosplenic territories of the reticuloendothelial system and of the hemopoietic tissue participate in the fixation of carbon granules. When doses greater than 16 mg per 100 g are injected we note an acceleration of the rate of blood cleansing, as a result of the intervention of a new factor which causes the retention of carbon in the lungs and other areas.

3. In the mouse the rate of disappearance of carbon from the blood is directly proportional to the amount injected, up to the dose of 32 mg per 100 g. The phenomenon of the acceleration of blood cleaning appears in this animal species only at doses that are essentially the double of those used in the rat. However, even at these high doses the phenomenon of acceleration of blood cleansing and the degree of pulmonary fixation of the carbon are more attenuated and irregular in the mouse than in the rat.

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